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Healing of Laser Incisions in Rat Dermis: Comparisons of the Carbon Dioxide Laser Under Manual and Computer Control and the Scalpel

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Background and Objective: **The Computer-Assisted Surgical Techniques (CAST) program was researched to decrease lateral tissue damage and improve wound healing subsequent to laser incision. CAST differs from the traditional laser because it makes the incision in a discontinuous manner, allowing tissue to cool during the incision process.**
Study Design/Material and Methods: **The transient temperature changes in the tissue adjacent to the incision were measured with a thermocouple in a rat model. The subsequent wound healing was studied with histology and tensiometry.**
Results: **The thermal measurements demonstrated that all CAST settings were cooler than the continuous mode of laser incision. However, histology and tensiometric studies showed mixed results.**
Conclusion: **This research demonstrates that CAST can be used in future surgical applications with no delay in wound healing as compared to the manually controlled laser. However, this study also finds no decrease in the wound healing time when using the CAST program. Lasers Surg Med 20:90–96, 1997.**

Key words: computer-controlled scanning; laser beam delivery; laser surgery; motion tracking; robotics

INTRODUCTION

The ability of lasers to provide precise tissue ablation has encouraged their implementation in a number of surgical and medical procedures. Considering the unique qualities of laser radiation, surgeons often view lasers as an alternative to the scalpel. In the operating room, lasers perform procedures where scalpels have limited or restrictive use. In addition, the hemostatic properties associated with the laser are often beneficial in the treatment of the patient.

With these benefits in mind, researchers have sought to improve the efficiency of the laser in medical applications. Several studies have reported impaired wound healing associated with laser-tissue interactions [1–7]. In contrast, other studies have described equivalent or better healing results when compared to scalpel wounds [8–11]. Regardless of which studies accurately describe the effects of the laser on wound healing,

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the lateral tissue damage caused by carbon dioxide (CO_2) laser ablation is indisputable.

In an attempt to reduce the time required for wound healing, this study seeks to determine if computer-assisted control of the laser would result in less lateral tissue injury due to a reduced temperature increase during the ablation process. The study investigated the thermal profile and wound healing properties of incisions created by a CO_2 laser controlled by the Computer-Assisted Surgical Techniques (CAST) program.

A CAST-controlled laser incision is made in a discontinuous manner by a computer directing the laser beam. Based on the results of incisions made in a gelatin model [12], CAST makes laser incisions at cooler temperatures because the step-wise motion of the laser beam allows tissue time to cool as the laser moves to new ablation sites. This study expanded the initial gelatin model results into an animal model to account for the more complex nature of living tissue and the effects of lateral temperature increases propagated by thermal diffusion and micro-circulation. The CAST incisions were compared to control scalpel incisions by an examination of tensile strength and histology.

MATERIALS AND METHODS

A Sharplan Model 1060™ (Sharplan, Allendale, NJ) 60W CO_2 laser was used at a continuous output of 5.0 W. The laser was focused to a 0.8 mm spot and directed on the tissue with a Microslad™ (Sharplan) micropositioner. The power output was chosen based on early trials of dermal incisions on the back of rats using a variety of settings from 1–15 W (data not shown). A preliminary histologic examination showed that higher power settings inflicted substantial lateral tissue damage, whereas lower power settings required an inordinate amount of time to create an incision with the necessary depth.

The laser beam was directed by mirrors on servos controlled by a LabView 2 (National Instruments, Austin, TX) program running on a Macintosh Quadra 840 AV computer (Apple Computer Co., Cupertino, CA). This program controls a Microslad™ (Sharplan) micropositioner that directs the beam path by two mirrors set in an X-Y plane. In Laserdraw, the LabView created menu, the surgeon can select a specific path pattern for the beam, as well as manipulate several parameters for the incision.

The CAST program creates an incision in a discontinuous manner as a series of spot ablations [12–14]. In other words, the laser ablates at a spot for 0.5 s, skips a preprogrammed distance, and ablates the new location for 0.5 s. The process is repeated until the beam moves the distance of the full incision. The laser then skips to the beginning of the incision and initiates the process again. To avoid irradiating the same spots on consecutive passes, a small offset in laser positioning is introduced at the beginning of each new pass using the randomize option in the Laserdraw menu. In this manner, a complete incision is created after several passes.

The step size is the distance between two consecutive spots, which can be altered to fit a particular need by using the Laserdraw menu. In investigating the thermal profile, tensile strength, and histology of these laser incisions, four different step sizes for CAST were used. Step sizes were measured as a function of laser spot size, which remains constant at 0.8 mm, TEM_{00} beam (Gaussian). The four step sizes investigated were continuous motion of the laser, and step sizes equal to 2, 3, and 6 times the spot size.

Temperature measurements were made by an Omega Type T (HYP-1, Omega, Stamford, CT) thermocouple connected to a Dash 8 (Astro-Med, West Warwick, RI) strip chart recorder. The thermocouple is in the tip of a 30 gauge needle (0.3 mm diameter). The temperature measuring system had a response time of < 2 ms, which ensured accurate temperature measurements. The temperature profile was plotted on a strip chart as a function of time. The thermocouple was used to record skin temperatures just lateral to the incision as the laser operated. Initially, the thermocouple was inserted 0.7 ± 0.1 mm from the planned path of ablation. This distance was measured manually using a ruler under a Zeiss (OPMI 1, Zeiss, Germany) surgical microscope. To provide a baseline skin temperature, the chart recorder was started for 5 s before the laser was used. The laser was then activated for 25 s, using the digital clock on the Dash 8 strip chart recorder. Because of minor amounts of skin contraction caused by tissue heating, the position of the thermocouple was measured after completion of the incision. The final distance from the tip of the thermocouple to the edge of the Helium-Neon targeting laser beam was measured and recorded. In these experiments, the values ranged from 0.2–1.2 mm. The strip chart recordings were analyzed to determine temperatures at 5, 10, 15, 20, and 25 s during the laser incision process. The values were recorded and categorized according to the step to spot ratio (con-

tinuous, 2×, 3×, 6×) as well as the final distance between thermocouple and the laser spot.

For the tensiometry and histology studies, 12 Sprague-Dawley rats were divided into four survival groups: 3, 7, 14, or 21 days. Four rats each were at the 3 and 7 day time points, whereas two rats each were at the remaining 14 or 21 day time points. Six incisions were made on each rat. Four incisions were made using CAST at different step/spot ratios: continuous, 2×, 3×, and 6×. Each rat served as its own control through the final two incisions: manually controlled laser and a #10 scalpel blade.

Each rat was anesthetized using a 10:1 mixture of Ketamine and Xylazine. The dorsal pelt was closely shaved to expose the skin. To prevent any gross motion of the target tissue during the ablation process, the dorsal pelt was sutured to a row of pins running lateral to the rat. Prior to making an incision, a 6″ scalpel guide was inserted subcutaneously in the fascial plane separating skin and muscle. The guide served to prevent the laser from ablating into the underlying muscle layer. After completing both the laser and scalpel incisions, the wound edges were approximated with two nylon sutures. Antibiotic ointment was applied to the wounds after surgery.

At each selected time point, wound tissue samples were harvested and placed in saline solution. Each wound was transversely cut into two separate samples. An ~3–4 mm strip of skin that contained the wound was fixed in 10% buffered-formalin for 72 hr at 4°C. The skin was then embedded in paraffin and sections (4 μm) were stained with hematoxylin and eosin or Masson's Trichrome using standard procedures. The histological analysis was performed by an evaluation of four criteria: wound epithelialization, amount of inflammation, area of granulation tissue, and area of eschar. In a blinded fashion, the samples were ranked from best to worst in for each criterion.

The remainder of each sample was tested for tensile strength (g/mm^2) by a tensiometer (Instron 1130, Canton, MA). Tensile strength was calculated from the maximal force placed perpendicular to the wounds before separation. The results were normalized with the measured wound width ~1.0 cm, and thus converted to tensile strength given a constant skin thickness. All wounds broke at strengths far below that of intact skin samples.

Data analysis and statistical analysis used a Macintosh computer and StatView (Abacus Concepts, Berkeley, CA). Data were plotted with Cricket Graph (Cricket Software, Malvern, PA).

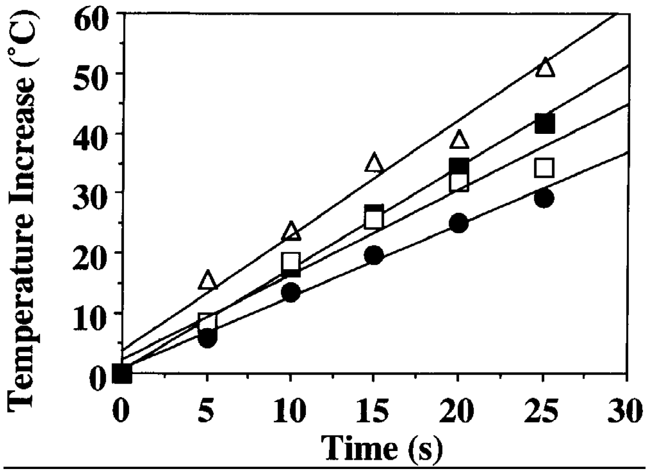

Fig. 1. Temperature increase as a function of time adjacent to an incision made with a CO_2 laser under computer control. The temperature values plotted are from T_{max} as explained in Eq. (1) in the text. The open triangles are obtained when moving the laser in a slow continuous motion across the skin. The closed squares are obtained when the laser is jumped with a step size equal to 2× the spot size. The open squares are from a step size equal to 3× the spot size. The closed circles are from a step size equal to 6× the spot size. The straight lines are linear least square fits to the data points.

RESULTS

Thermocouple Study

Figure 1 demonstrates the difference between a continuous incision and three CAST settings. The continuous incision has higher maximum temperatures than any of the three CAST settings. The raw data consist of 34 measurements at five time points from six animals. The spatial distribution of temperature from any given set of incisional parameters was fit with a Gaussian as given in equation (1)

$$y = T_{max} \exp[-(x^2)/\alpha^2] \quad (1)$$

Where T_{max} is the temperature at the incision. The value of T_{max} is determined from the fit of the individual data points. Equation (1) is a Gaussian distribution and accurately describes the temperature distribution in a homogenous material. We use this distribution of temperature for the dermis as a first-order approximation. In this model, the coefficient α describes to the width of the temperature distribution and is related to the thermal diffusion coefficient and the heat capacity of the skin. In the initial analysis, α was a free parameter and found to be equal or near 0.5 mm (within the errors of the fits). We, therefore, set α

= 0.5 mm in the analysis of all the data and reduced fluctuations in T_{max}.

In Figure 1, we plot T_{max} versus the time for the continuous incisions and three step sizes using CAST. The overall trend has been calculated to be statistically significant (simple regression) by using a linear least-squares fit to determine the time dependence of the temperature. These linear fits are also shown on Figure 1. The slopes of the linear fits were listed as a function of the step size (where the continuous incision was given a step size that was one-third times the spot size). The slopes of the time dependence of the temperature increase inversely correlated with the step size with a P value $\leqslant 0.031$.

Tensiometric Study

In Figure 2a, we show the tensile strength of all the incisions as a function of time. Any values that are statistically different from the others are marked with an asterisk ($P \leqslant 0.05$, Bonferroni/Dunn multiple comparison). All the incisions made by the laser had a significantly smaller tensile strength than those made by the scalpel. Also, all the laser incisions showed no statistically significant difference in tensile strength for the different settings of the CAST system (except for a step size of 3 × spot size on day 3). Figure 2 is a result of an average of four animals per 3 and 7 day time points and two animals per 14 and 21 day time points, with a set of six incisions on each animal. In Figure 2b, we look at the average tensile strength of the laser incisions averaged for days 7, 14, and 21. The trend of the weaker tensile strength with step size is statistically significant with a $P \leqslant 0.05$ using a one factor analysis of variance (ANOVA). Since tensile strength is normally used to differentiate wounds in the later steps of healing, we did not include the day 3 wounds in these averages.

Histological Study

Figure 3 demonstrates the results from the histologic studies. The histological rankings were tallied ranging from one to ten, with the best rank in each category being a ten. Thus the incisions that rank the best have the largest average score. The results are derived from two animals for each time point. Time points of 3 days, 7 days, and 14 days were used, and each animal had all laser incisions with scalpel incisions serving as controls. The control scalpel incisions constantly ranked the best (scored the highest) in each category, which obstructs the comparison between the various laser wounds. Therefore, the scalpel controls are not shown in the histological graph to better visualize the differences between the different CAST settings. Histologically, there is little difference between the wounds on day 21, so data from that day were not included in the average score.

The plot shows the average histological scores with all four criteria equally weighted. The error bars represent the standard errors of the means. The observed trend of the histological average score decreasing with step size from continuous to 6 × spot size was shown to be significant with a one factor ANOVA that results in a $P \leqslant 0.002$.

DISCUSSION

The investigations presented here were initiated by the results of a preliminary study using a collagen gel model. Transient temperature measurements in the collagen gel demonstrated that the CAST program produced incisions with lower lateral temperatures when compared to a manually controlled laser. On the basis of these results, a similar procedure was investigated using a live animal model. The goal was to investigate the effects of microcirculation on heat production and on thermal diffusion and subsequent wound healing. The results of the thermal profiles in the animal model indicated that the three CAST step sizes had significantly lower lateral temperatures than the continuously stepped laser incision. The results in the animal model confirmed the results from the gelatin model and prompted the next phase of the experiments.

The investigations involving the histology and tensiometry were performed to determine if the lower temperatures produced by the CAST system resulted in an improvement in tensile strength and general healing. We note that scalpel incisions consistently showed much better healing than any of the laser incisions. However, when we compare only those incisions made by the laser, we do observe some trends. The results indicate that despite the production of lower temperatures, wounds produced by the CAST system with a small step size showed no significant increase in tensile strength when compared to the manually controlled laser. In fact, a statistically significant decrease of tensile strength was noted for the largest step size. To show a significant result, the tensile strengths at three time points (days 7, 14, and 21) were averaged. Since the 21-

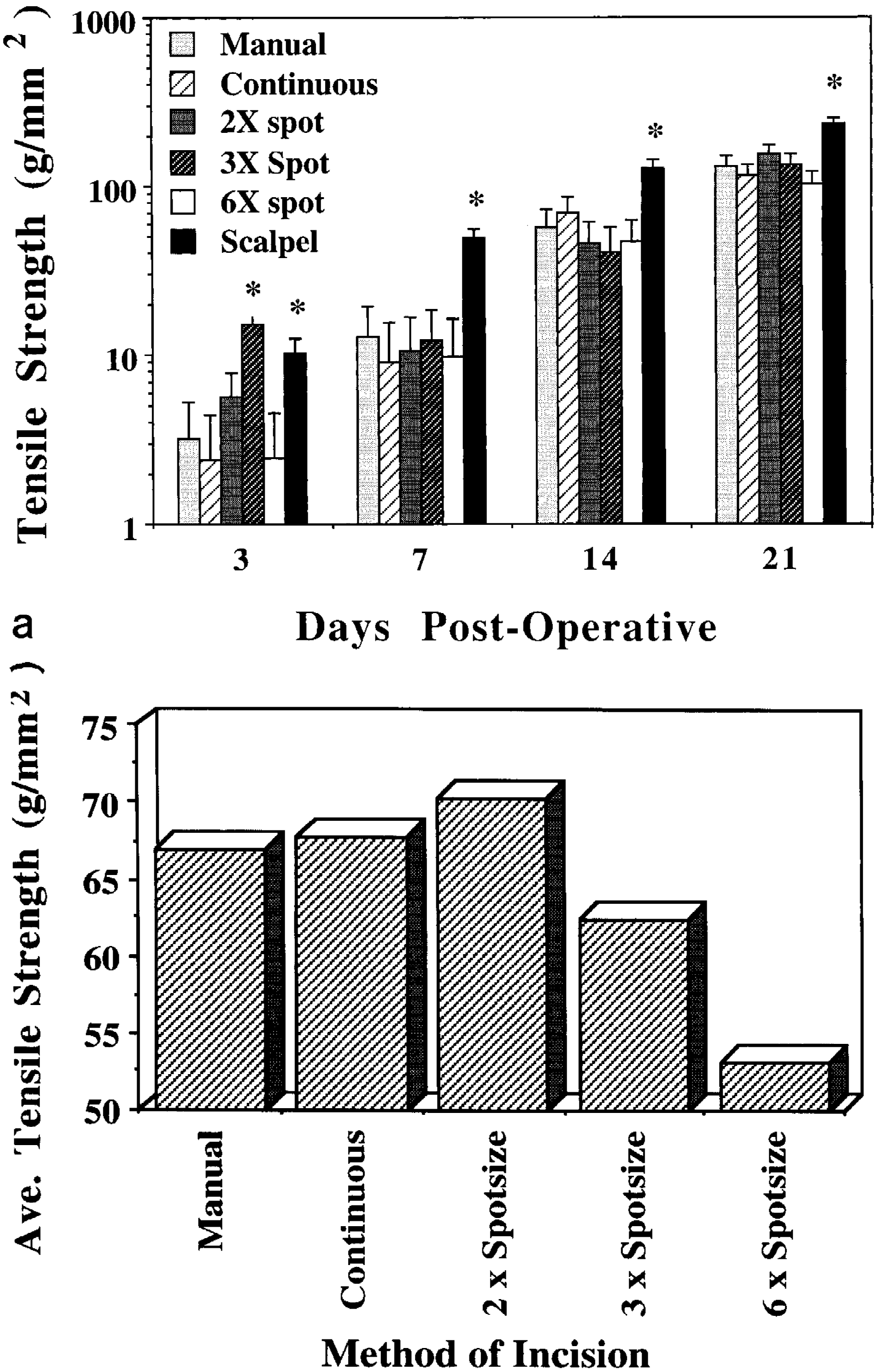

Fig. 2. (**a**) Comparison of the wound tensile strength at postoperative days 3, 7, 14, and 21. The key showing designating the method of creating the incision is shown. Statistically significant differences in tensile strength are designated with an asterisk. Note the vertical axis is a log axis. (**b**) Comparison of the average tensile strength of the laser incisions from days 7, 14, and 21.

day wounds were the strongest, they had the most influence on the averages. We averaged data from different days, because we are trying to establish overall differences in the methods. However, we lose all temporal information in this analysis.

In analyzing the histology, we averaged the

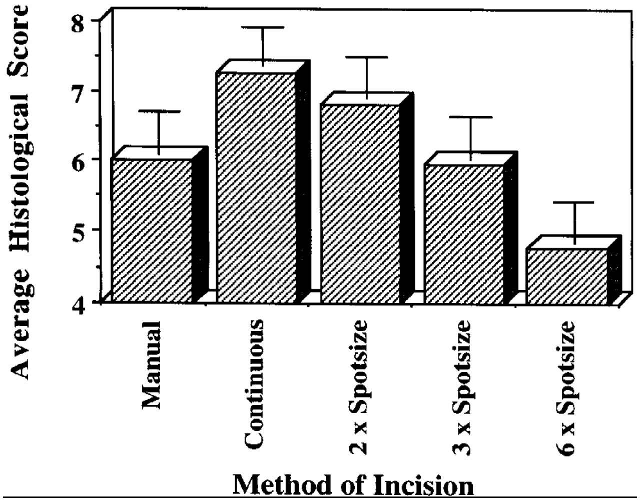

Fig. 3. The average histologic scores of the laser incisions from days 3, 7, and 14. The higher scores correlate with better healing. The error bars represent standard errors of the means.

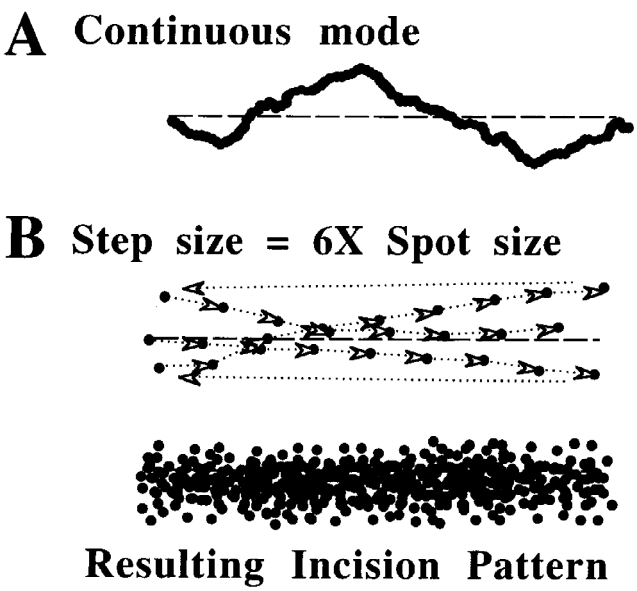

Fig. 4. A schematic sketch showing the effect of motion on the laser incisions. In **A**, the laser is moved in a continuous mode when making the incision. In **B**, the laser is jumped with a step size of ~ 6 × the spot size. The resulting incision with a scatter of laser irradiation points is also shown.

results from three time points (days 3, 7, and 14). It is reasonable that tensile strength is used to evaluate wounds that are nearly completely healed (21 days postincision) and the histology is used earlier in the healing process (days 3–14). The day 21 histology is hampered by a reduction in the number of criteria used to determine a healing hierarchy. At 3 weeks into the wound repair, healing in all the samples had progressed to the point where epithelialization was complete and the eschar and inflammation had disappeared. The only criterion for comparison was the amount of granulation tissue on day 21.

The average histology scores for the laser incisions as shown in Figure 3 follow approximately the same trend as the tensile strengths in Figure 2b. The small step size CAST incisions were histologically better than the manual incisions with a significant decrease in the histological score for the largest step size.

Although the transient temperature changes, the tensiometry and the histology seem to give conflicting results, they are consistent when one takes into account the manner in which the body heals laser wounds. The transient increase in temperature indicates the incision will suffer from some lateral tissue damage. The smaller temperature transients will create slightly less lateral damage. Yet in all cases, the temperature was high enough for cell necrosis. In the repair process, the body must remove all the damaged tissue in the early steps of the healing process. The time required by the body to remove slightly different volumes of damaged tissue is roughly the same. Thus a small decrease in the volume of damaged tissue does not significantly decrease wound healing times.

Thus the tensile strength shows no improvement in wound strength using CAST with a small step size in comparison to a manual incision. The histology does show a small improvement. This is because the histology was ranked on the area of damage, among other criteria. So, the decrease in the transient temperature change and the resulting decrease in the volume of lateral tissue damaged showed higher histological scores.

The worsening of wound repair, both histologically and in the tensiometry, with large step size (six times the spot size) is harder to understand. We believe it is due to motion of the target during the incision process. The animals used in these experiments were living, breathing animals with motions from respiration and pulse. In addition, tissue contraction from thermal transients moves the incision during the ablation process. In Figure 4a, we schematically show how these motions might distort an incision made with a laser moving along in a continuous manner. The resulting incision is not a straight line but a line with a thickness of approximately the laser spot size. In Figure 4b, we schematically show how the motions affect an incision made with a large step size

in a very different manner. The resulting incision is approximately a straight line, but it suffers from a “shotgun” effect of laser incision sites. The width of tissue irradiated with the laser is much wider than the laser spot size. We believe this problem of tissue motion must be solved by tracking the target and moving the laser beam to compensate for the tissue motion.

CONCLUSIONS

Laser incisions show delayed wound healing over scalpel incisions. Part of the delay in wound healing is due to the lateral thermal damage caused by the laser. We have used the CAST system to reduce the lateral temperature increase and thereby reduce lateral tissue damage. This improvement is apparent in the histological analysis of wound healing. However, it is not clearly observed in the tensiometry data. As a surgical system, CAST offers significant advantages over the manually controlled laser. In instances where precision or repetition is required, a CAST system can be implemented with excellent results. Because the CAST system with small step sizes matches the manually operated laser in wound healing performance, it can be used preferentially in instances that best fit its various strengths. For example, CAST holds the advantage over a manually directed laser in its ability to make an incision with a preset pattern and uniform depth. CAST’s discontinuous manner of forming incisions needs to be combined with motion tracking to produce a laser that can compensate for movement during the ablation process. When movement occurs during the laser incision, the computer would compensate after a few milliseconds and place the laser beam back on the original incision track. Regular movement such as breathing must be predicted and the computer must move the laser accordingly. Without this tracking, we observe no significant decrease in the wound healing times for rat dermal incisions.

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REFERENCES

1. Madden JE, Edlich RF, Custer JR, Panek PH, Thul J, Wangensteen OH. “Studies in the management of the contaminated wound. Resistance to infection of surgical wounds made by knife, electrosurgery and laser.” Am J Surg 1970; 119:222–224.
2. Hall RR. The healing of tissues incised by a carbon dioxide laser. Br J Surg 1971; 58:222–225.
3. Hashimoto K, Rockwell RJ, Epstein RA, Fidler JP. Laser wound healing compared with other surgical modalities. Burns 1971; 1:13–16.
4. Cochrane JPS, Beacon SP, Creasy GH, Russell CG. Wound healing after laser surgery: An experimental study. Br J Surg 1980; 67:740–743.
5. Fisher SE, Frame JW, Browne RM, Tranter RMD. Comparative histological study of wound healing following CO_2 laser and conventional surgical excision of canine buccal mucosa. Arch Oral Biol 1983; 28:287–291.
6. Durkin GE, Duncavage JA, Toohill RJ, Tieu TM, Caya JG. Wound healing of the true vocal cord squamous epithelium after CO_2 laser ablation and cup forceps stripping. Otolaryngol Head Neck Surg 1986; 95:273–277.
7. Loumanen M, Meurman JH, Lehto VP. Extracellular matrix in healing CO_2 laser incision wound. J Oral Pathol 1987; 16:322–331.
8. Tauber C, Fairne I, Horoszowski H. Healing of CO_2 laser incision in the skin and fascia. Harefuh 1980; 98:1–3.
9. Norris CW, Mullarsky MD. Experimental skin incisions made with the carbon dioxide laser. Laryngoscope 1982; 92:416–419.
10. Finterbush A, Rousso M, Ashur H. Healing and tensile strength of CO_2 laser incisions and scalpel wounds in rabbits. Plastic Reconstr Surg 1982; 70:360–362.
11. Robinson JK, Garden JM, Taute PM, Leibovich SJ, Lautenschlager EP, Hartz RS. Wound healing in porcine skin following low-output carbon dioxide laser irradiation of the incision. Annals Plastic Surg 1987; 18:499–505.
12. Reinisch L, Mendenhall MH, Charous S, Ossoff RH. Computer-assisted surgical techniques using the Vanderbilt free electron laser. Laryngoscope 1994; 104:1323–1329.
13. Reinisch L, Mendenhall MH, Ossoff RH. Medical delivery systems for the Vanderbilt free electron laser. In: Harrington JA, Harris DM, Katzir A, Milanovich FP eds. “Biomedical Fiber Optic Instrumentation.” SPIE 2131, pp 266–277.
14. Ossoff RH, Reinisch L. Computer assisted surgical techniques: A vision for the future of otolaryngology-head and neck surgery. J Otolaryngol 1994; 23:354–359.